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Brosinski, Katrin ; Gutbrod, Andreas ; Venzin, Claudio ; Grest, Paula

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**What is your diagnosis? Peritoneal fluid from a 3.5-year-old spayed dog with abdominal pain**

KATRIN BROSINSKI[1], ANDREAS GUTBROD[2], CLAUDIO VENZIN[2], PAULA GREST[1]

[1]Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Switzerland

[2]Department for Small Animal Surgery, Vetsuisse Faculty, University of Zurich, Switzerland

**Corresponding author:**

Katrin Brosinski

Institute of Veterinary Pathology, Vetsuisse Faculty

University of Zurich, Winterthurerstr. 268

CH-8057 Zurich, Switzerland

Tel.: +41-44-6358589

Fax.: +41-44-6358934

Email: k.brosinski@access.uzh.ch

**Case Presentation**

A 3.5-year-old, spayed female dachshund was presented to the Department for Small Animal Surgery of the University of Zurich for sudden apathy, increasingly painful abdomen, ascites, mild fever, pale mucous membranes, and reduced body condition. Serum biochemistry profile and CBC abnormalities included mild, nonregenerative anemia (hematocrit, 33 %, reference interval 42-55 %; MCH 21 pg, reference interval 23-26 pg; MCV 60 fl, reference interval 64-73 fl; slight anisocytosis and poikilocytosis of erythrocytes), mild neutrophilia ($9.29x10^3$/µl; reference interval $2.5\text{-}7.4x10^3$/µl), mild hypoproteinemia (protein, 54 g/L; reference interval, 56-71 g/L), mild hypoalbuminemia (albumin, 23 g/L; reference interval, 29-37 g/L), mildly increased alkaline phosphatase (113 U/L; reference interval 20-98 U/l) and aspartate-amino-transferase activity (119 U/l, reference interval 20-44 U/l). Abdominal ultrasonographic examination revealed a small amount of free fluid and a large midabdominal cavernous, 9 cm in diameter large mass suspected to be located in the right branch of the pancreas, displacing the stomach and the duodenum cranially and to the left. A sample of peritoneal fluid was collected, fine needle aspiration of the mass was performed and both were sent for cytological examination (Figure 1). The abdominal fluid was serosanguinous and cloudy. The nucleated cell count was 208250/µl and the total protein concentration was 36 g/l (refractometric determination).

**Cytologic findings**

Smears of the abdominal fluid and the mass were comparable. Both were highly cellular. There were about 90 % neutrophils, 9 % macrophages, and few mesothelial cells and lymphocytes. Most neutrophils were nondegenerated. In the background there were moderate numbers of basophilic, membrane-like, folded structures with a width of 60-100 µm and embedded barely stained, globular or crystal-like structures, which were interpreted as calcareous corpuscules (Figure 1). The membranes stained intensely with Periodic acid-Schiff stain (PAS). The cytologic findings were interpreted as suppurative inflammation of the liver and pyoabdomen, with intralesional parasitic structures, consistent with *Echinococcus* sp..

**Additional results**

Exploratory ventral midline laparotomy revealed a hepatic mass in the right caudal lobe rather than a pancreatic mass. A brittle, whitish, multicystic mass measuring 9 cm in diameter was present (Figure 2). The other liver lobes appeared normal and a lobectomy was performed. Sections for histology were prepared using standard methodology (formalin fixation and hematoxylin and eosin, PAS) and were examined (Figure 3).

Histologically, the hepatic mass consisted of a large multilocular cyst, that replaced the hepatic parenchyma. The cyst wall was formed by an outer layer of granulation tissue with admixed numerous neutrophils and epitheloid cells as well as few eosinophils and multinucleated giant cells surrounding necrotic cell debris and multiple collapsed and ruptured, up to 5 mm diameter cysts. These cysts were lined by a 10-20 μm wide eosinophilic, hyaline outer membrane layer and an inner lightly basophilic layer containing numerous 5-20 μm diameter, basophilic calcareous corpuscules. A severe multifocal granulomatous hepatitis with multiloculated, hydatid cyst consistent with *Echinococcus* sp. was diagnosed and confirmed by PCR using *E. multilocularis*-specific primers.[1]The dog recovered well from surgery and clinical signs resolved.

**Discussion**

The finding of membrane-like folded structures that are intensely PAS positive in cytological preparations is highly characteristic for hydatid cysts and therefore ultrasound-guided fine needle aspiration cytology (FNAC) is considered as an inexpensive, and fast tool in establishing human echinococcosis.[2,3] However, detection of such membrane-like structures on FNAC has not been described in veterinary medicine even though they are easily demonstrated in cytologic preparations from infected animals.

Echinococcal cysts in dogs develop most frequently in the liver and lungs, but other organs can be affected.[3,4] Cysts can be identified by diagnostic imaging as a multilocular mass and typical parasitic membranes can be demonstrated by FNAC. Larval stages of other cestodes, namely *Mesocestoides* sp. and *Taenia* sp. are possible differential diagnoses. However, larvae of *Mesocestoides* sp. do not form cysts and therefore no membranes will be found. Metacestodes of *Taenia pisiformis* and *hydatigena* may be found in the liver and abdominal cavity of dogs, but form small and unilocular cysts up to 1 cm in diameter formed by multilayered thick (100-200 μm) membranes in contrast to the large and multilocular cyst formed by thin membranes found in the presented case. If alveolar echinococcosis is suspected based on cytological findings it might be confirmed by examination of tissue samples. Species identification in tissue sections is possible because membranes of *E. granulosus* are usually thicker (150-200 μm) than the ones of *E. multilocularis* (5-15 μm);[5,4] however, PCR is the preferred method for definitive identification.[1,3]

In our institution nine cases of alveolar echinococcosis (eight dogs, one non-human primate) were diagnosed cytologically between 2001 and 2010. Two of these were confirmed histologically as *E. multilocularis*. Our case report demonstrates that ultrasound guided FNAC is a useful tool in diagnosing canine alveolar echinococcosis.

Veterinary diagnosticians should be aware of the possible occurrence of these characteristic parasitic structures in cytological smears.

**Key Words:** Abdominal fluid, cytology, Echinococcus multilocularis

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**Declaration**

The authors declared that they had no conflicts of interests with respect for their authorship of the publication of this article.

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**Figures**

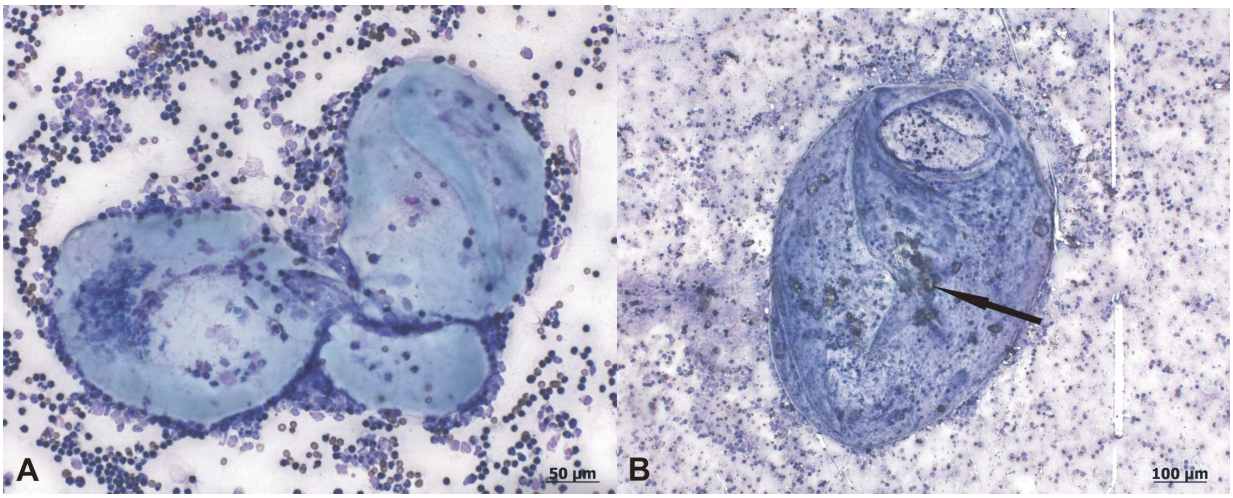


**Figure 1.** Direct smear from peritoneal fluid from a dog. Modified Wright`s. (**A**) x 20 objective (**B**) Calcareous corpuscles (arrow). x 10 objective.

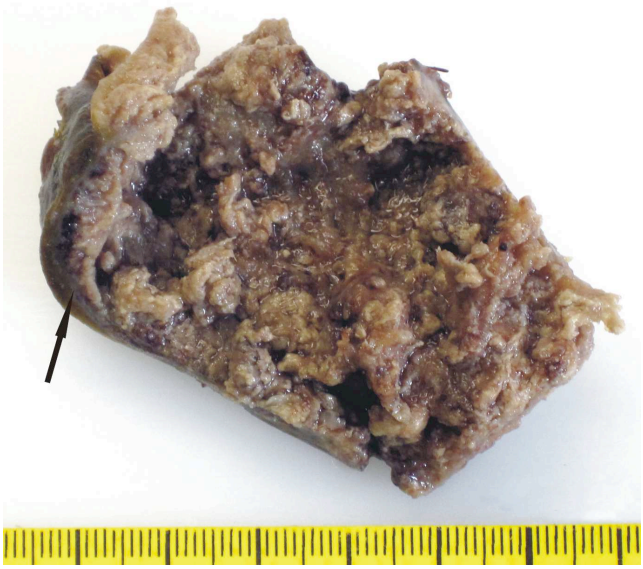

**Figure 2.** Macroscopic view on a part of a hepatic parasitic cyst with a thick capsule (arrow) and an irregular inner surface.

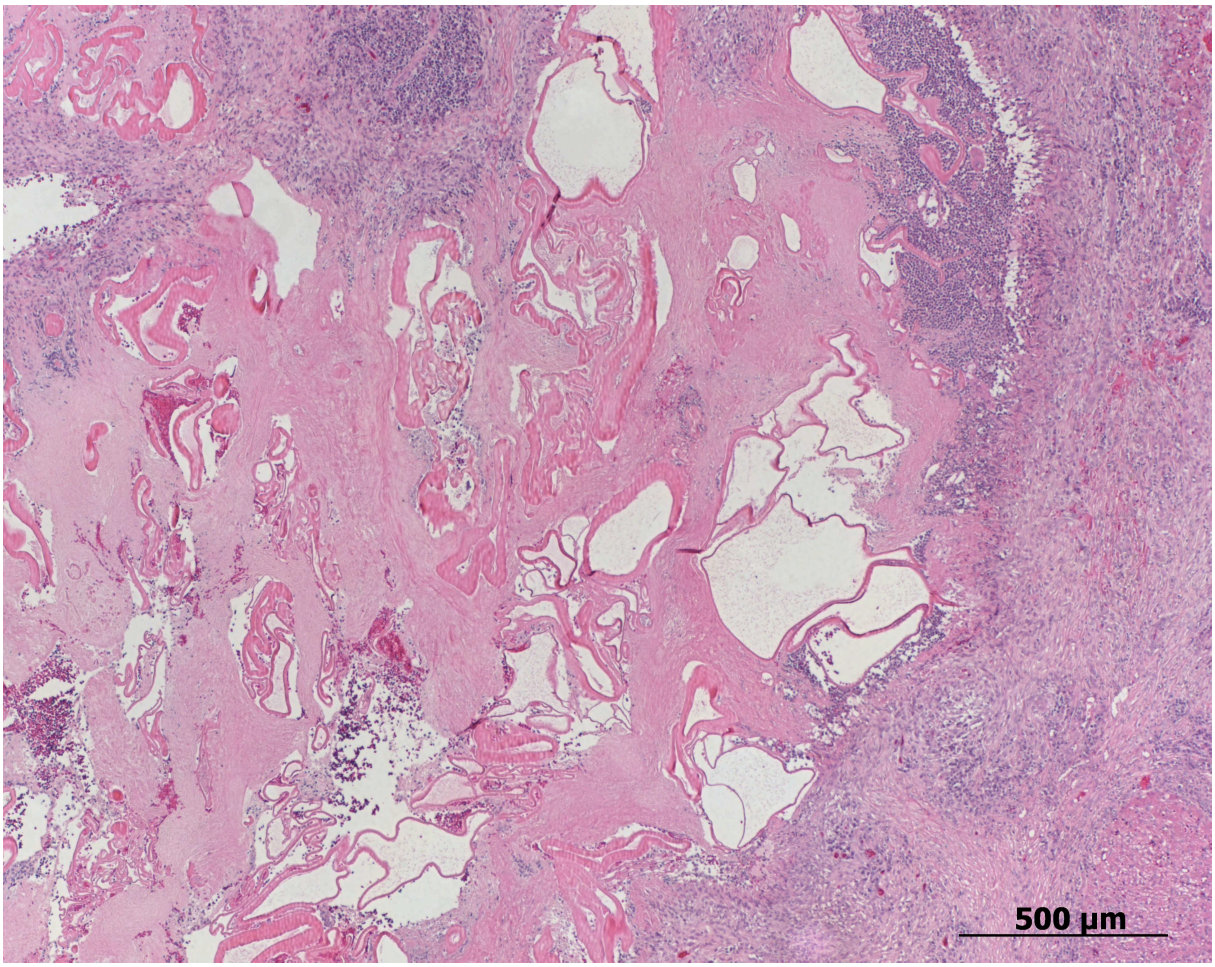


**Figure 3.** Liver: Histopathologic section of multiple parasitic cysts, surrounded by a severe chronic granulomatous inflammation. H&E. 4 x objective.

Request reprints from Katrin Brosinski, Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 268, CH-8057, Zurich, Switzerland. Email: k.brosinski@access.uzh.ch